

Factors Influencing Cadmium Accumulation and Its Toxicity to Marine Organisms

by David W. Engel* and Bruce A. Fowler†

The toxicity of dissolved cadmium to a variety of marine animals has been found to be related to salinity, with decreased toxicity observed at higher salinities. Recent data from our laboratory have demonstrated that the toxicity of cadmium to estuarine shrimp and larval fish is a function of free cadmium ion concentration, which in turn is controlled by the chloride concentration of the water. As the chloride concentration (i.e., salinity of the water) increases, the concentration of free cadmium ion decreases relative to total dissolved metal, due to its complexation with chloride ions. These observations have been given further support by measurements involving the uptake of ^{115m}Cd by shrimp which showed that accumulation of ^{115m}Cd and chloride concentration also are inversely related.

Experiments also have been conducted on the physiological effects of cadmium on the respiration of excised oyster gill tissue. Although tissues from oysters exposed for 14 days to 0.1 ppm total dissolved cadmium accumulated significant quantities of metal, no measurable effects on respiration rates were detected. Higher doses (0.3 and 0.6 ppm) caused both mortalities of oysters and accelerated respiration of excised oyster gill. Exposure to 0.1 ppm cadmium also caused the induction of and/or increased binding of cadmium to a specific low molecular weight protein in oysters. This protein appeared to have a detoxification function at low cadmium exposure levels, but in animals exposed to 0.6 ppm cadmium the induction mechanism apparently became saturated, allowing the excess cadmium to bind critical sites with resultant damage.

Introduction

In recent years, it has become evident that in order to understand the impact of anthropogenic additions of trace metals to the aquatic environment, it is of prime importance to also understand the chemical and physiological processes which control the accumulation of these metals by organisms. Cadmium is of importance because of its use in various industrial processes and as a by-product of zinc mining. Both of these types of activities have resulted in elevated concentrations both in saline waters and in marine organisms. In order to better understand the potential effects of cadmium on marine organisms, our discussions will be based on an understanding of the chemistry of

cadmium in saline media. The discussions will center upon the chemical speciation of cadmium; how speciation is related to bioavailability, and toxicity; and some of the physiological and biochemical responses of oysters to accumulated cadmium.

Chemical Speciation

In the past, accumulation and toxicity investigations on cadmium have been conducted with little attention to the chemical form of the metal in the experimental media. This lack of regard for the chemistry of cadmium may be the source for much of the variability in the data published on cadmium toxicity to estuarine and marine organisms (Table 1). Thus, it seems reasonable that before we can understand the effects of cadmium on marine organisms, we must understand its chemistry in natural waters, including the effect of environmental factors on chemical form.

Recently information has been obtained which

* NOAA, National Marine Fisheries Service, Southeast Fisheries Center, Beaufort Laboratory, Beaufort, North Carolina 28516.

† National Institute of Environmental Health Sciences, Laboratory of Environmental Toxicology, Research Triangle Park, North Carolina 27709.

indicates that the toxicity of copper and cadmium in water is dependent upon the concentration of the free metal ion in the medium. Sunda and Guillard (1) and Anderson and Morel (2) demonstrated that cupric ion activity, rather than the concentration of total dissolved copper, was the causative agent in copper toxicity to algae. Similar results concerning copper toxicity on the freshwater cladoceran, *Daphnia magna*, have been published by Andrew, Biesinger, and Glass (3) and for cadmium and the grass shrimp, *Palaemonetes pugio* (4). These experiments used both organic and inorganic ligands to control the activities of cupric and cadmium ion in the test media. Thus, it appears that in many instances trace metal availability and toxicity is a function of free metal ion whose concentration may be significantly less than the total dissolved metal concentrations, depending upon the level of complexation.

The chemistry of cadmium was investigated in different dilutions of seawater to gain a better understanding of the relationship between cadmium availability (free ion versus total dissolved metal) and toxicity (4). The measurements of cadmium ion concentrations were made at constant dissolved metal concentrations with a cadmium ion-selective electrode. The data showed that free cadmium ion varied inversely with salinity due to complexation

by chloride ion (Fig. 1). Also, inorganic chemical speciation models based on equilibrium calculations predict that cadmium is primarily present as chloride complexes in seawater (5). Recently, Mantoura, Dickson, and Riley (6) published computed models for the complexation of trace metals by inorganic ions and humic type materials in natural waters. Their calculations support our observations on the complexation of cadmium by chloride in sea water dilutions. They found that humates complexed only slightly with cadmium, even under the most favorable conditions and that chloride complexes dominated the cadmium speciation in estuarine waters at salinities above 20 ‰. Thus, in any toxicological, biochemical, or physiological procedure where cadmium is used, the chemical composition of the medium must be known before the true toxicity of cadmium can be evaluated.

Toxicity of Cadmium

Data on the toxicity of cadmium to aquatic organisms indicates that freshwater organisms are more sensitive to cadmium than are marine organisms (7, 8) and that the relationship between toxicity and salinity is inverse, i.e. as the salinity increases the toxicity of cadmium decreases (4, 9-11). These results may be explained by the model discussed earlier if the toxicity of cadmium, as with copper, is dependent upon the concentration of free ion in the water. To test this hypothesis, a series of experiments was conducted to examine cadmium toxicity as a function of free cadmium ion (4).

In these experiments the grass shrimp, *Palaemonetes pugio*, was chosen as the test organism. The shrimp were exposed for four days to a range of salinities of from 5 to 30‰ and a range of total cadmium concentrations of from 1.1×10^{-6} to $5.9 \times 10^{-6}M$. Measurements of free cadmium ion concentration were made directly in the experimental media using a cadmium ion-selective electrode.

Examination of the four-day survival data for the grass shrimp showed that as the salinity decreases there is a corresponding decrease in survival at all levels of cadmium used (Fig. 2). Similar results have been interpreted in terms of the interaction between cadmium and damage to a salinity-dependent physiological function, such as osmoregulation (10). However, when the data are correlated with cadmium ion concentration and salinity ($p[Cd^{2+}]$, $(-\log \text{ of the cadmium ion concentration rather than total dissolved metal})$ a striking relation between free ion concentration and survival emerges (Fig. 3). Thus, the observed salinity effect on cadmium toxicity to grass shrimp can be explained entirely in terms of the free cadmium ion as it is affected by

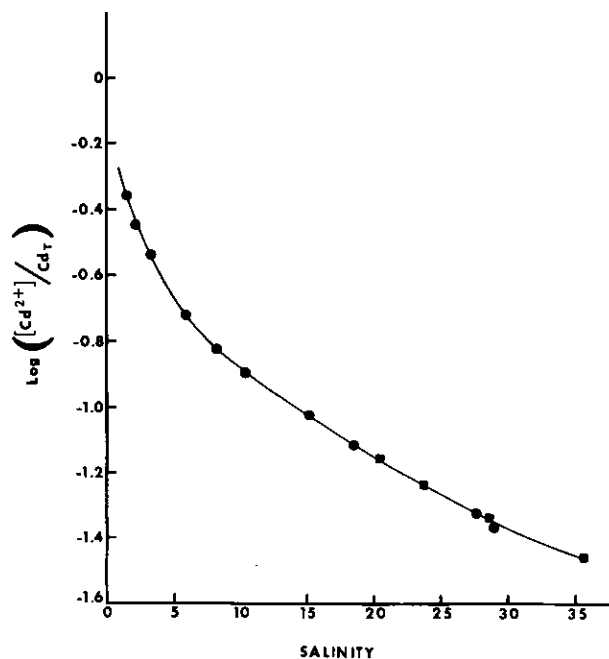


FIGURE 1. Logarithm of the fraction of total cadmium present as free cadmium ion as a function of salinity. $[Cd^{2+}]$ refers to free cadmium ion concentration and Cd_t to total dissolved cadmium. Figure from Sunda, Engel, and Thuotte (4).

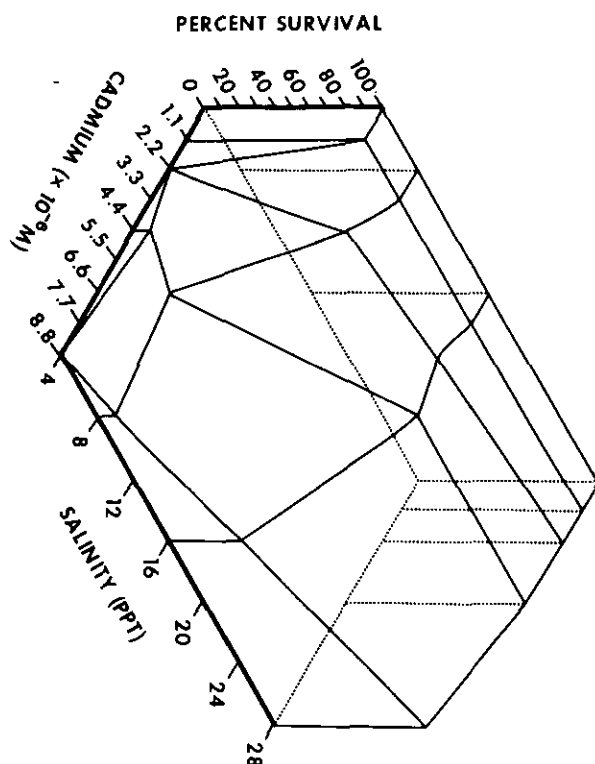


FIGURE 2. Three-dimensional plot of survival of grass shrimp, *Palaemonetes pugio*, exposed to four different salinities and five different cadmium concentrations for 96 hr.

chloride complexation. In order to demonstrate that free cadmium ion and not salinity was the major independent variable, the cadmium ion concentration was varied independent of salinity (5‰) using different concentrations of cadmium and a chelator NTA (nitrilotriacetic acid). Again, survival was related to free cadmium ion, $p[Cd^{2+}]$ (Fig. 3). The close replication of the relationship between survival and $p[Cd^{2+}]$ in the two separate experiments demonstrated that the free cadmium ion was the toxic chemical form. The rapid decrease in survival over narrow $p[Cd^{2+}]$ range indicates a rather sharp endpoint for the titration of cadmium onto biologically sensitive sites. From these data it seems reasonable to predict that in the marine environment for a given concentration of cadmium it should be most toxic to organisms living in the upper portions of estuaries where the salinities are the lowest.

A comparison of published cadmium toxicity data (4, 15), recalculated in terms of $p[Cd^{2+}]$, with estimated concentrations of free cadmium in seawater ($p[Cd^{2+}] \geq 10.5$), suggest that cadmium is not environmentally significant from the acute toxicity standpoint (Table 1). All of the calculated values for these data are four to five orders of magnitude lower than the $p[Cd^{2+}]$ calculated for the environment.

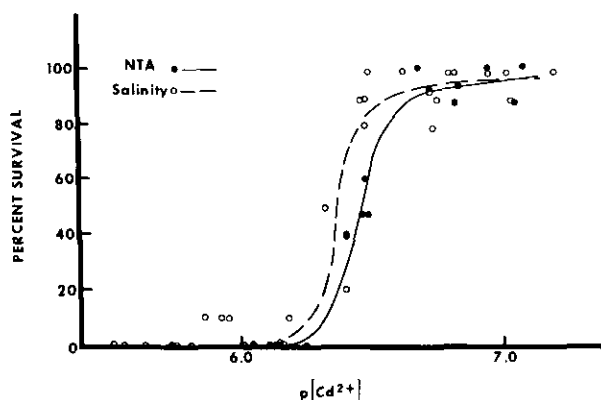


FIGURE 3. Plot of the four-day survival of grass shrimp, *P. pugio* as a function of $p[Cd^{2+}]$ ($-\log$ of free Cd^{2+} concentration). The data for both the salinity and NTA (nitrilotriacetic acid) were replotted from Sunda, Engel, and Thuotte (4).

However, most toxicity determinations were made on adults and therefore did not necessarily test the most sensitive life stages of organisms.

For chronic or protracted exposures, generalizations concerning toxic effects of cadmium in the environment should not be made. The primary difficulty is that there are not sufficient data from long-term experiments to either refute or support any general statements concerning biological impacts. An apparent exception is the work of Nimmo et al. (16), who showed that by using the life-cycle bioassay technique and the mysid shrimp, *Mysidopsis bahia*, it was possible to demonstrate significant effects at cadmium concentrations of $10.6 \mu g/l$. at about 20‰ salinity in a 17 day life-cycle test. They also demonstrated changes in the breeding cycle and reductions numbers of young released. The calculated $p[Cd^{2+}]$ for these results is 8.2, which is much closer to the calculated environmental value. Through the use of either long-term exposure or life-cycle bioassays a more valid estimation of cadmium toxicity in the marine environment may be made.

Accumulation

Cadmium is actively accumulated by marine organisms, particularly by mollusks (17-20), and some species of mollusks can accumulate large quantities of metals from contaminated environments with no apparent damage. Two examples are *Mytilus edulis*, which accumulated up to 60 ppm of cadmium (21) and the Pacific oyster, *Crassostrea gigas*, which has been shown to accumulate cadmium up to 120 ppm on a dry weight basis (22). In laboratory investigations the American oyster, *Crassostrea virginica*, has been shown to be capable of concen-

Table 1. Calculated $p[\text{Cd}^{2+}]$ values from cadmium LC_{50} for selected marine organisms.^a

Organism	Salinity, ‰	Time, hr	LC_{50} , ppm Cd	$p[\text{Cd}^{2+}]$	Reference
Sand shrimp <i>Crangon septemspinosa</i>	20	96	0.32	6.71	(8)
Grass shrimp <i>Palaemonetes vulgaris</i>	20	96	0.42	6.60	(8)
Grass shrimp <i>Palaemonetes pugio</i>	15	96	0	6.36	(4)
Soft-shell clam <i>Mya arenaria</i>	20	96	2.2	5.88	(8)
Blue mussel <i>Mytilus edulis</i>	20	96	25.0	4.82	(8)
Sand worm <i>Nereis virens</i>	20	96	11.0	5.18	(8)
Mummichog <i>Fundulus heteroclitus</i>	20	96	55.0	4.48	(8)
Sheepshead minnow <i>Cyprinodon variegatus</i>	20	96	50.0	4.52	(8)
Mummichog (larvae) <i>F. heteroclitus</i>	30	48	23-7.8	5.1-5.5	(12)
Silverside (larvae) <i>Menidia menidia</i>	30	96	0.6	6.65	^b
Oyster (embryo) <i>Crassostrea virginica</i>	25	48	3.8	5.7	(13)
Bay scallop <i>Argopecten irradians</i>	25	96	1.48	6.13	(14)

^a Calculated by equation of Sunda et al. (4): $-\log[\text{Cd}^{2+}] = -\log[\text{Cd}_{\text{total}}] - \log[\text{Cd}^{2+}/\text{Cd}_{\text{total}}]$.

^b D. W. Engel, unpublished data.

trating high levels of cadmium (17, 19), and whole body concentrations about 100 ppm wet weight were fatal (21).

To gain further insight into the effect of salinity on cadmium availability, experiments were conducted with ^{115m}Cd to test the effect of salinity on rates of cadmium accumulation. The organism used in these experiments was the grass shrimp, *Palaemonetes pugio*, which were exposed to ^{115m}Cd for four days in water of 5, 10, 20, and 30 ‰. The uptake by the shrimp was inversely related to salinity (Fig. 4). The pattern follows the model of free ion concentration shown in Figure 1; i.e., at constant total cadmium, as the salinity increases the concentration of free cadmium ion decreased. Even though the time period allowed for uptake of ^{115m}Cd from the water was short, it was equivalent to the period previously used for the toxicity tests (4).

Preliminary investigations with the accumulation of cadmium by the oyster, have shown that it was necessary to allow exposed oysters 24 hr in flowing seawater to clear the mantle cavity and gut before metal concentrations were measured. This period of flushing allowed the oyster to depurate cadmium which was not biologically incorporated. Qualitative information on the distribution of cadmium on the mantle has been obtained from oysters exposed to 0.1 ppm cadmium for 14 days and then flushed for

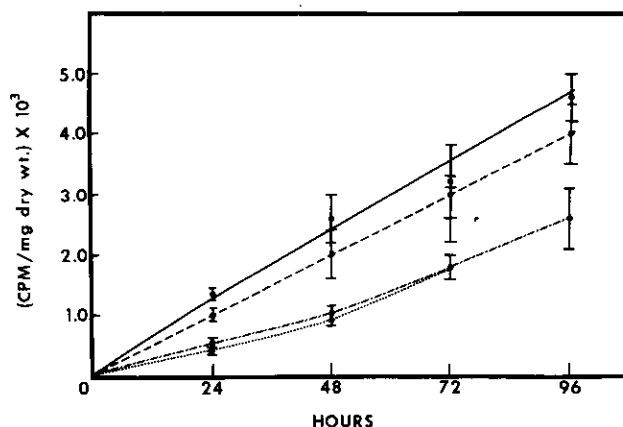


FIGURE 4. Accumulation of ^{115m}Cd by grass shrimp, *P. pugio*, as a function of time of exposure and salinity: (—) 5‰; (---) 10‰; (· · ·) 20‰; (- · -) 30‰. The vertical bars represent \pm SE for $n=5$.

24 hr in clean water. By scanning freeze-dried mantle tissue with a PIXEA (proton-induced x-ray emission analysis) unit, the concentrations of cadmium on the surface of the tissue were shown to increase as the scan moved toward the mouth from the outer edge (Fig. 5). This observation indicated that cadmium was probably adsorbed by the mucus coat on the gills and the mantle and then was moved toward the mouth by ciliary action. If the oysters

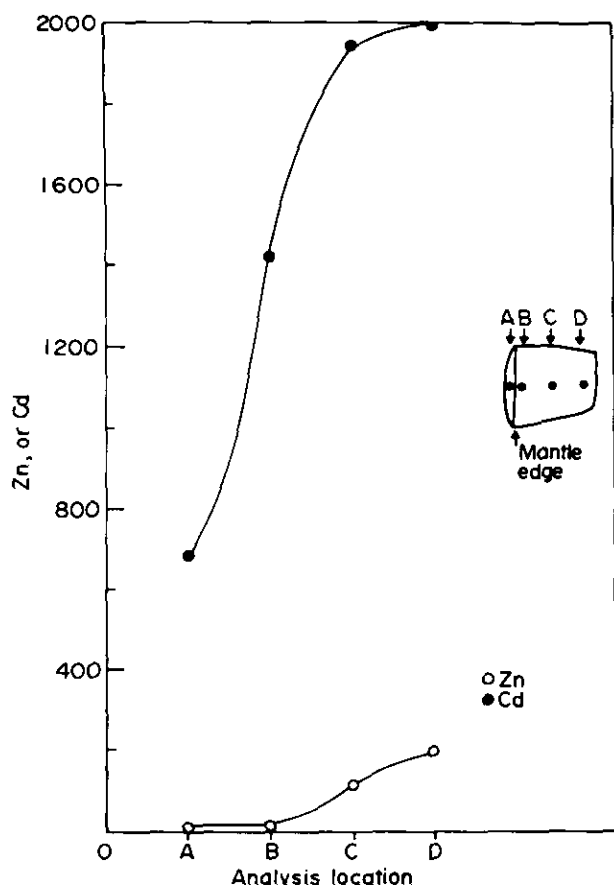


FIGURE 5. Proton-induced x-ray emission analysis (PIXEA), microscan of a mantle from an oyster exposed to 0.1 ppm cadmium in seawater for 14 days.

are not allowed to clear in flowing water, large quantities of unbound cadmium would be included in tissue samples, which would result in unrealistically high accumulation values and increased variability.

In another series of experiments, juvenile oysters, *Crassostrea virginica*, were exposed to ^{115m}Cd at three salinities, 10, 20, and 30 ‰ for 7 days. In these accumulation experiments, the oysters were removed from the radioactive water at predetermined intervals and placed in nonradioactive water for 24 hr. The flushing period was used to allow the oysters time to clear the unassimilated ^{115m}Cd that was in the gut and adsorbed to external surfaces and mucus. The remaining ^{115m}Cd is most probably "incorporated" rather than nonspecifically adsorbing to surfaces. The animals were then killed and prepared for liquid scintillation counting. The soft parts were placed in vials, dried, weighed and digested with concentrated HNO_3 . After the samples were taken to dryness they were covered with 10 ml of a standard toluene base cocktail and counted in a

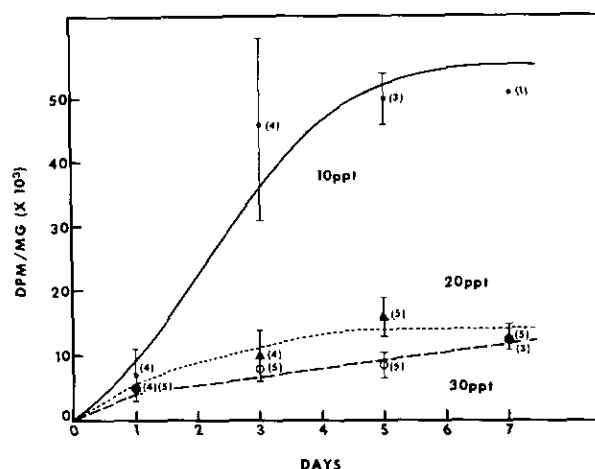


FIGURE 6. Accumulation of ^{115m}Cd by juvenile American oysters, *Crassostrea virginica*, as a function of time of exposure and salinity of the water. The numbers in parentheses refer to the number of individuals per point \pm SE. Juvenile oysters supplied by Frank M. Flowers and Sons, Inc., Bayville, N. Y.

scintillation counter. This technique gave reproducible counting geometry.

The uptake of the ^{115m}Cd by the oysters as a function of salinity follows a similar pattern, as was observed for the grass shrimp (Fig. 6). A major difference is that the exposure period was continued for a long enough time, 7 days, so that maximum uptake occurred at a salinity of 10 ‰. The mortality of oysters at 10 ‰ probably was caused by the toxic effect of cadmium, because the total cadmium concentration was ~ 1.0 ppm in each exposure tank. This concentration of stable cadmium was the result of cadmium "carrier" in the isotope stock, rather than from an added spike of cadmium. While this level is not toxic at 20 or 30 ‰, it apparently was at 10 ‰ due to the greater availability of free cadmium ion.

Both of the experiments help explain the environmental observation that organisms collected along a salinity gradient down an estuary toward the ocean have decreased levels of cadmium as the salinity increased (18, 22). Such observations fit the model of increased cadmium availability with decreased salinity. All of the accumulation data which have been collected thus far have involved only uptake from the water, but other patterns could develop if the primary source of the metal was from food. This is an area which should receive extensive research in the future.

Physiological Effects

Effects of cadmium on the physiological balance of marine organisms have been demonstrated by

numerous investigators (17, 24-27). However, the following discussion will be confined to the mollusks, more particularly to results of our investigations with the American oyster, *Crassostrea virginica*.

The oysters used in these investigations were exposed to cadmium in a flowing water exposure system for 14 days (17). The concentrations of cadmium which were used were 0.1, 0.3, and 0.6 ppm cadmium in 3.0-3.4% seawater, which corresponds to $p[Cd^{2+}]$ levels of from 7.4 to 6.7. Measurements of oxygen consumption rates were made on excised gill section using a differential respirometer. Measurements of total cadmium were made on the same tissue sections as used for respiration determinations. The tissues were wet ashed in concentrated HNO_3 and analyzed by atomic absorption spectrophotometry.

The rate of accumulation of cadmium by the gills

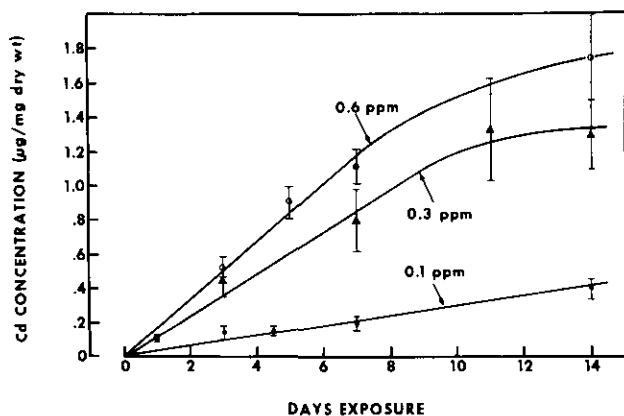


FIGURE 7. Accumulation of cadmium by oyster gill tissue as a function of duration of exposure (14 days) and cadmium concentration (0.1, 0.3, and 0.6 ppm). The vertical bars show \pm SE and $n=10$ at 0.1 and 0.6 ppm and $n=5$ at 0.3 ppm. Data for 0.1 and 0.6 ppm from Engel and Fowler (17).

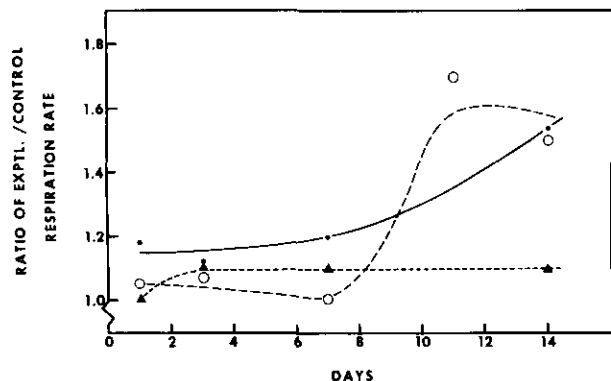


FIGURE 8. Normalized respiration rates of excised oyster gill tissue to cadmium concentrations of (▲) 0.1, (○) 0.3, and (●) 0.6 ppm (17).

of exposed oysters was both concentration- and time-dependent (Fig. 7). Cadmium uptake by the gill tissue was found to level off at about 11 days in the oysters exposed to 0.6 and 0.3 ppm cadmium. Accumulation of cadmium at 0.1 ppm was virtually linear through day 14, and there were significant differences between the rates of uptake of cadmium at 0.6 and 0.3 ppm and 0.1 ppm. Also, there were mortalities among the oysters exposed to either 0.3 and 0.6 ppm cadmium by day 14, but no mortalities occurred among those exposed to 0.1 ppm cadmium or the controls.

Oxygen consumption of oyster gill tissue was affected by the accumulated cadmium after about 7 days, when the animals were stressed (Fig. 8). These data have been recalculated (17) for the purpose of showing the possible relationship between oxygen consumption and the accumulated metal. Since all of these measurements were not done at the same time, it is difficult to make any hard comparisons, but from the experiments at 0.3 and 0.6 ppm cadmium there appears to be an upper cadmium tissue concentration above which the animal can not metabolically control. Such speculation is supported by Shuster and Pringle (23), who demonstrated that at body burden of about 100 ppm cadmium wet weight of oysters were killed, and that the maximum concentration in the oyster was independent of dose rate. Thus, apparently the detoxification system for cadmium in oysters must have some

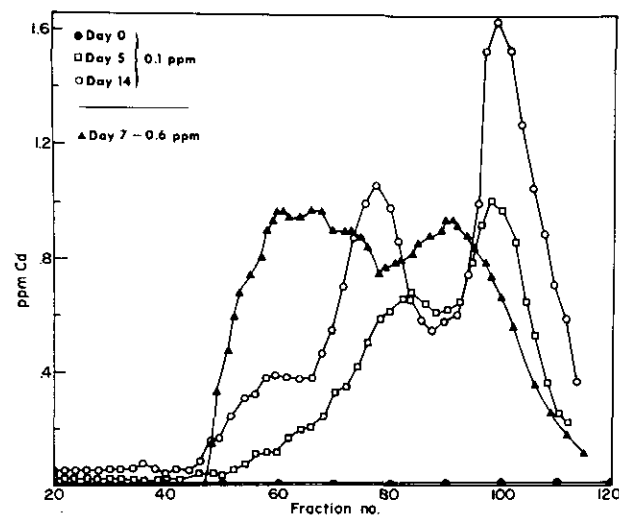


FIGURE 9. G-75 Sephadex elution profile from oysters exposed to 0.1 ppm cadmium for (□) 5 and (○) 14 days, and (▲) from oysters exposed to 0.6 ppm for 7 days, relative to (●) controls. Curves show association of cadmium with low molecular weight proteins (0.1 ppm) and with high molecular weight proteins (0.6 ppm). Techniques used for isolation and characterization of the cadmium-binding protein are given in Ridlington and Fowler (31).

point of saturation.

The capacity of marine organisms to detoxify materials which have been accumulated should be of selective advantage. Ridlington and Fowler (28) have already demonstrated the oyster possesses a possible detoxification system for cadmium in the form of a low molecular weight metal-binding protein. Cadmium binding proteins have been isolated from other marine organisms, primarily fish and seals (29,30). The oyster cadmium-selective protein has a molecular weight of about 7400 and has high concentrations of dicarboxylic amino acids relative to cysteine (31). Therefore, the chelation of the cadmium may be through the carboxyl groups rather than the sulfhydryls as in vertebrate metallothioneins. Further examinations of the oyster protein indicate that there is an upper limit beyond which the oyster cannot sequester and thereby detoxify the accumulated cadmium (Fig. 9). This phenomenon can be explained by the spill-over of cadmium into the higher molecular weight fraction in oysters exposed to 0.6 ppm cadmium for 7 days. Such an ill-defined pattern indicates that all the available protein has combined with cadmium, and that additional metal will complex with sensitive sites on membranes or enzyme molecules. Whether the cadmium selective protein is produced *de novo*, or whether the protein is simply activated by the presence of the metal is unknown. The first option is the more attractive but it is very difficult to prove, due to technical problems in measuring protein synthesis in a bivalve mollusk. However, from an evolutionary standpoint, an inducible gene to produce a detoxifying protein would be of selective advantage. Further investigations are currently in progress to examine other factors which may influence the uptake and toxicity of cadmium in the marine environment.

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REFERENCES

1. Sunda, W., and Guillard, R. R. L. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *J. Mar. Res.* 34: 511 (1976).
2. Anderson, D. M., and Morel, F. M. N. Copper sensitivity of *Gonyaulax tamarensis*. *Limnol. Oceanogr.* 23: 283 (1978).
3. Andrew, R. W., Biesinger, K. E., and Glass, G. E. Effects of inorganic complexing on the toxicity of copper to *Daphnia magna*. *Water Res.* 11: 309 (1977).
4. Sunda, W. G., Engel, D. W., and Thuotte, R. M. Effect of chemical speciation on toxicity of cadmium to grass shrimp, *Palaemonetes pugio*: Importance of free cadmium ion. *Environ. Sci. Technol.* 12: 409 (1978).
5. Zirino, A., and Yamamoto, S. A pH-dependent model for the speciation of copper, zinc, cadmium and lead in seawater. *Limnol. Oceanogr.* 17: 661 (1972).
6. Mantoura, R. F. C., Dickson, A., and Riley, J. P. The complexation of metals with humic materials in natural waters. *Estuarine Coastal Mar. Sci.* 6: 387 (1978).
7. Negilski, D. S. Acute toxicity of zinc, cadmium and chromium to marine fishes, yellow-eye mullet (*Aldrichetta forsteri* C. & V.) and small-mouthed hardy-head (*Atherinopsoma microstoma* (Whitley)). *Austral. J. Mar. Freshwater Res.* 27: 137 (1976).
8. Eisler, R. Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. *J. Fish. Res. Board Can.* 28: 1225 (1971).
9. Rosenberg, R., and Costlow, J. D. Synergistic effects of cadmium and salinity with constant and cycling temperatures on the larval development of two estuarine crab species. *Mar. Biol. (Berl.)* 38: 219 (1976).
10. Jones, M. B. Synergistic effects of salinity, temperature and heavy metals on mortality and osmoregulation in marine and estuarine isopods (Crustacea). *Mar. Biol. (Berl.)* 30: 13 (1975).
11. Westernhagen, H. von, Rosenthal, H., and Sperling, K.-R. Combined effects of cadmium and salinity on development and survival of herring eggs. *Helgol. Wiss. Meeresunters* 26: 416 (1974).
12. Middaugh, D. P., and Dean, J. M. Comparative sensitivity of eggs, larvae and adults of the estuarine teleosts, *Fundulus heteroclitus* and *Menidia menidia* to cadmium. *Bull. Environ. Contam. Toxicol.* 17: 645 (1976).
13. Calabrese, A., et al. The toxicity of heavy metals to embryos of the American oyster, *Crassostrea virginica*. *Mar. Biol. (Berl.)* 18: 162 (1973).
14. Nelson, D. A., et al. Biological effects of heavy metals on juvenile bay scallops, *Argopecten irradians*, in short term exposures. *Bull. Environ. Contam. Toxicol.* 16: 275 (1976).
15. Boyle, E. A. The marine geochemistry of trace metals. Ph.D. thesis, Massachusetts Institute of Technology, 1976.
16. Nimmo, D. R., et al. The acute and chronic effects of cadmium on the estuarine mysid, *Mysidopsis bahia*. *Bull. Environ. Contam. Toxicol.* 19: 80 (1978).
17. Engel, D. W., and Fowler, B. A. Copper and cadmium induced changes in the metabolism and structure of molluscan gill tissue. In: *Marine Pollution: Functional responses*. W. B. Vernberg, T. Calabrese, and F. Thurberg, Eds., Academic Press, New York, in press.
18. Huggett, R. J., Bender, M. E., and Slone, H. D. Utilizing metal concentration relationships in the eastern oyster (*Crassostrea virginica*) to detect heavy metal pollution. *Water Res.* 7: 451 (1973).
19. Eisler, R., Zarogran, G. E., and Hennekey, R. J. Cadmium uptake by marine organisms. *J. Fish. Res. Board Can.* 29: 1367 (1972).
20. Brooks, R. R., and Rumsby, M. G. Studies on the uptake of cadmium by the oyster, *Ostrea sinuata* (Lamarck). *Austral. J. Mar. Freshwater Res.* 15: 53 (1967).
21. Nickless, G., Stenner, R., and Terrille, N. Distribution of cadmium, lead and zinc in the Bristol Channel. *Mar. Pollut. Bull.* 3: 188 (1972).
22. Ayling, G. M. Uptake of cadmium, zinc, copper, lead and chromium in the Pacific oyster, *Crassostrea gigas* grown in the Tamar River, Tasmania. *Water Res.* 8: 729 (1974).
23. Shuster, C. N., Jr., and Pringle, B. H. Trace metal accumulation by the American eastern oyster, *Crassostrea vir-*

- ginica*. Proc. Natl. Shellfish Assoc. 59: 91 (1969).
24. Vernberg, W. B., et al. Effects of sublethal concentrations of cadmium on adult *Palaemonetes pugio* under static and flow-through conditions. Bull. Environ. Contam. Toxicol. 17: 16 (1977).
 25. Dawson, M. A., et al. Physiological response of juvenile striped bass, *Morone saxatilis*, to low levels of cadmium and mercury. Chesapeake Sci. 18: 353 (1977).
 26. Thurberg, F., and Dawson, M. A. Physiological response of the cunner, *Tantagolobrus adspersus*, to cadmium. III. Changes in osmoregulation and oxygen consumption. U. S. Dept. Commerce Natl. Mar. Fish. Serv. Spec. Sci. Rep. Fish. 681: 11 (1974).
 27. Thurberg, F., Dawson, M. A., and Collier, R. S. Effects of copper and cadmium on osmoregulation and oxygen consumption in two species of estuarine crabs. Mar. Biol. (Berl.) 23: 171 (1973).
 28. Ridlington, J. W., and Fowler, B. A. Isolation and partial characterization of an inducible cadmium-binding protein from the American oyster. Pharmacologist 19: 180 (1977).
 29. Olafson, R. W., and Thompson, J. A. J. Isolation of heavy metal binding proteins from marine vertebrates. Mar. Biol. (Berl.) 28: 83 (1974).
 30. Brown, D. A. Increases of Cd and Cd:Zn ratio in the high molecular weight protein pool from apparently normal liver of tumor-bearing flounders (*Parophrys vetulus*). Mar. Biol. (Berl.) 44: 203 (1977).
 31. Ridlington, J. W., and Fowler, B. A. Isolation and partial characterization of a cadmium-binding protein from the American oyster (*Crassostrea virginica*). Chem. Biol. Interact., in press.